

Species traits and abundances predict metrics of plant–pollinator network structure, but not pairwise interactions

Colin Olito and Jeremy W. Fox

C. Olito (colin.olito@gmail.com) and J. W. Fox, Dept of Biological Sciences, Univ. of Calgary, 2500 University Dr. NW, Calgary, AB, T2N 1N4, Canada.

Plant–pollinator mutualistic networks represent the ecological context of foraging (for pollinators) and reproduction (for plants and some pollinators). Plant–pollinator visitation networks exhibit highly conserved structural properties across diverse habitats and species assemblages. The most successful hypotheses to explain these network properties are the neutrality and biological constraints hypotheses, which posit that species interaction frequencies can be explained by species relative abundances, and trait mismatches between potential mutualists respectively. However, previous network analyses emphasize the prediction of metrics of qualitative network structure, which may not represent stringent tests of these hypotheses. Using a newly documented temporally explicit alpine plant–pollinator visitation network, we show that metrics of both qualitative and quantitative network structure are easy to predict, even by models that predict the identity or frequency of species interactions poorly. A variety of phenological and morphological constraints as well as neutral interactions successfully predicted all network metrics tested, without accurately predicting species observed interactions. Species phenology alone was the best predictor of observed interaction frequencies. However, all models were poor predictors of species pairwise interaction frequencies, suggesting that other aspects of species biology not generally considered in network studies, such as reproduction for dipterans, play an important role in shaping plant–pollinator visitation network structure at this site. Future progress in explaining the structure and dynamics of mutualistic networks will require new approaches that emphasize accurate prediction of species pairwise interactions rather than network metrics, and better reflect the biology underlying species interactions.

Mutualistic interactions between plants and their animal pollinators exhibit tremendous diversity in their nature and specificity, ranging from highly specialized species pairings to indiscriminate generalists (Jordano 1987, Waser et al. 1996). At the community level, this variation emerges as a network of mutualistic interactions specifying both who pollinates whom, and who provides food resources for whom. Studies of pollination interaction networks have increasingly focused on identifying causal links between species' traits and the detailed structure of species interactions in order to predict the ecological and evolutionary dynamics of plants and their animal pollinators (Strauss and Irwin 2004, Vázquez et al. 2009a, Junker et al. 2010, 2013).

Pollination interaction networks exhibit highly conserved structural properties across diverse habitats, species assemblages, and time (Jordano et al. 2003, Vázquez et al. 2009a). Interaction networks are sparse (a small fraction of all possible interactions are actually observed, i.e. connectance is low), nested (specialized species tend to interact with a proper subset of the species with which generalists interact), exhibit skewed 'degree distributions' (most plants are pollinated by only a few pollinators, and most pollinators pollinate only a few plants), and species are significantly more specialized than expected given the availability of potential mutualistic

partners (Jordano et al. 2003, Blüthgen et al. 2008, Dupont et al. 2009). The generality of these network structural properties suggests general underlying mechanisms (Jordano et al. 2003), and recent network studies have emphasized the need to test putative determinants of network structure, such as species traits and abundances (Vázquez et al. 2007, 2009b).

Several hypotheses for underlying determinants of network structure have been supported by empirical and theoretical work, the most successful of which are the neutrality and biological constraints hypotheses. Under the neutrality hypothesis, species interact randomly, resulting in interaction frequencies proportional to relative species abundances (Vázquez and Aizen 2004, Canard et al. 2014). The biological constraints hypothesis posits that constraints imposed through trait mismatches between potential mutualists strongly influence network structure (Jordano et al. 2003, Santamaria and Rodriguez-Girones 2007). It is important to note that both neutral and trait-based models constrain predicted network structure by identifying interactions that are impossible or unlikely due to low local abundances, trait mismatches, or both. Studies that have evaluated both hypotheses suggest that metrics of network structure like connectance and nestedness are best predicted by a combination of neutral interactions and various biological

constraints, especially mismatches in species spatio-temporal distributions (Jordano et al. 2006, Kallimanis et al. 2009, Vázquez et al. 2009b), species traits (Stang et al. 2007, 2009, Junker et al. 2010, 2013, Ibanez 2012, Ságayo et al. 2013; but see Vizentin-Bugoni et al. 2014), and phylogeny (Verdú and Valiente-Banuet 2011).

However, prediction of metrics of network structure may not stringently test the neutrality and biological constraints hypotheses. Many network metrics are insensitive to small changes in network topology (Fox 2006), covary strongly (Kay and Schemske 2004, Vermaat et al. 2009, James et al. 2012), and are sensitive to sampling effects (Dorado et al. 2011, Rivera-Hutinel et al. 2012). For example, Vázquez et al. (2009b) used species abundance, locality, and phenology data to predict several metrics of network structure and species pairwise interactions. They found that plant–pollinator interaction probabilities calculated from relative species abundances and phenologies provided sufficient information to generate random networks with some structural metrics that were indistinguishable from those of the observed network (nestedness, interaction asymmetry), but not to predict the observed pairwise interactions. More recent studies using similar methods have also enjoyed success predicting network metrics while struggling to predict pairwise interactions (Verdú and Valiente-Banuet 2011, Ságayo et al. 2013, Vizentin-Bugoni et al. 2014). This contrast suggests that existing models may predict network structural metrics well, but for the wrong reasons. Insofar as neutral interactions, and biological constraints like phenological mismatches, really determine which pollinators pollinate which plants, they should correctly predict specific pairwise interactions, not just metrics of network structure.

This result may reflect the network metrics being predicted. Vázquez et al. (2009b), Ságayo et al. (2013) and Vizentin-Bugoni et al. (2014) considered only metrics calculated for qualitative networks, which record only whether a given pollinator species pollinates a given plant species. Metrics calculated for quantitative networks that record the frequencies of different plant–pollinator interactions are more sensitive to small changes in network structure, and more robust to sampling effects (Blüthgen et al. 2008, Almeida-Neto and Ulrich 2011). Whether current statistical models of network structure can successfully predict measures of both qualitative and quantitative network structure is unknown.

The disparity in predictive ability of network models also may result from the practical challenges of sampling network data (Hegland et al. 2010). For example, under-sampled species may appear more specialized than they really are (Blüthgen et al. 2008, Dorado et al. 2011). For relatively rare species, incomplete sampling may result in unexpected mismatches between species observed interaction frequencies and theoretical interaction probabilities.

Few datasets include the data necessary to evaluate the effects of multiple biological mechanisms on both metrics of network structure and specific pairwise interactions. In particular, most reported plant–pollinator interaction networks lack phenological data and so lack the ability to evaluate phenological constraints (Vázquez et al. 2009b). Consequently, whether neutral interactions or biological constraints correctly capture important determinants of

network structure remains unclear. Here, we address these questions with a new, temporally explicit alpine plant–pollinator interaction network, and associated species abundance and morphology data. We assess neutral interactions and putative biological constraints as predictors of both network structural metrics and specific pairwise interactions. Additionally, we examine the effect of incomplete sampling of rare interactions on the performance of these models. We discuss implications for network models designed to predict detailed network structure, and the biological interpretation of network structural metrics.

Methods

Study system and sampling methods

We studied the plant–pollinator assemblage in a large, contiguous low-alpine meadow on the east face of Mt Murray (50°45′24.2″N, 115°17′07.2″W), located in the Canadian Rockies in Kananaskis Country, Alberta, during summer 2010. We sampled interactions between plants and pollinators in a square 1-ha plot located at 2350–2410 m elevation on every day that weather conditions were suitable for pollinator flight, from the day of first flowering, until killing frosts occurred and pollinators were no longer observed (24 June 2010–26 August 2010, a total of 32 sampling days). That is, we sampled on as many days as was biologically possible. We recorded plant–pollinator interactions along three 2 × 100 m transects between the lower and upper plot boundaries. An interaction was recorded when an insect visitor was observed contacting floral reproductive structures. We documented interactions between 41 flowering plant species and 125 insect species, and constructed a quantitative plant–pollinator interaction matrix, with rows and columns corresponding to plant and pollinator species respectively, and cell values indicating the number of visits observed between corresponding plant and pollinator species.

All network data, as well as a more detailed description of the study system and sampling methods are available as an online supplement (Supplementary material Appendix 1–2). Network data is also available from the Dryad Digital Repository <<http://doi.org/10.5061/dryad.7st32>>.

We independently estimated floral density per m² for plant species on each sampling day in five 1-m² quadrats placed randomly along the same transects. Pollinator species abundances were not estimated independently, but using the sum of all observed interactions for each species (Vázquez et al. 2005, 2007). All analyses used floral density as an estimate of plant-species abundance because individual flowers are the most natural resource unit for pollinators, and because flower density accounts for variability in floral display size among individuals of the same species.

All plant and pollinator species were assigned to one of four size classes based on flower depth, and proboscis length respectively. Assignments were made to approximately correspond such that the position of pollen and/or nectar best matched the proboscis length of potential pollinators in the same size class. Plant species were assigned to one of four morphology classes based species descriptions in Moss (2008), and field observations. Classes consisted of 1) disk

florets, 2) small, 3) medium, and 4) large corolla/nectar spur. Animal species were assigned to morphology classes based on proboscis length. For each species, the length of the extended proboscis for a representative sample (> 10 individuals whenever possible) of specimens was measured using calipers, or under a dissecting microscope using an ocular micrometer, after softening dried specimens in a relaxation chamber. For rare (and often extremely small) species, proboscis length could not be determined without destroying the specimen. These species were assigned to the closest size class based on genus/species descriptions and visual inspection under a dissecting microscope (see Supplementary Material Appendix 1 for genus/species descriptions references). Animal size classes consisted of 1) < 2 mm, 2) 2–4 mm, 3) 4–9 mm, or 4) > 9 mm proboscis length.

Network models

To evaluate the contributions of neutral interactions, species phenology, and morphology as determinants of network structure, we constructed interaction-probability models from species abundance, phenological, and phenotypic data (model abbreviations and descriptions in Table 1). Following the methods described in Vázquez et al. (2009b), we formulated models of all species pairwise interaction probabilities from vectors or matrices of species traits or relative abundances. We use matrix notation, where subscripts p indicate plants, a indicate animals, and $'$ indicates the vector or matrix transpose. We constructed models using vectors of total seasonal relative abundances ($\mathbf{N} = \mathbf{n}_p \mathbf{n}_a'$), species pairwise temporal overlap data ($\mathbf{T} = \mathbf{O}_{tp} \mathbf{O}_{ta}'$, where \mathbf{O}_t are binary matrices of species occurrence with rows for each species, and columns for each sampling day), and species morphology classifications ($\mathbf{M} = \mathbf{m}_p \mathbf{m}_a'$, where \mathbf{m} are matrices of morphology classifications; species can only interact with potential mutualists in the same class).

We also formulated several additional models that modified assumptions made by the neutrality and biological constraints hypotheses. These included a time-structured abundance model ($\mathbf{Nt} = \mathbf{n}_p \mathbf{n}_a'$, where \mathbf{n} are matrices of species daily relative abundances) to account for variation in species relative abundances over time; a continuous-phenology model (\mathbf{Tc}), in which species phenologies spanned all days between their first and last observation; and a morphology cascade model \mathbf{Mc} in which pollinators can interact with any plant of the same or smaller size class.

Table 1. Model descriptions.

Model abbreviation	Model description
N	Seasonal relative abundances
Nt	Daily relative abundances
T	Daily occurrence
Tc	Daily occurrence, first to last observation inclusive
M	Pollinators can interact with plants of the same size class only
Mc	Pollinators can interact with plant of the same or smaller size class

Notes: Combined probability models are calculated as the element-wise products between different combinations of the above models, normalized to sum to 1.

Models incorporating combined interaction probabilities resulting from multiple biological constraints can be formulated as the element-wise product of these single-factor models, which is then normalized to sum to one. We combined these single-constraint models to create a total of two two-constraint models (**NT**, **NTc**, **NM**, **NMc**, **TM**, **TMc**, **TcM**, **TcMc**, **NtM**, **NtMc**), and four three-constraint models (**NTM**, **NTMc**, **NTcM**, **NTcMc**). Lastly, we formulated a null model, **NULL**, in which all-pairwise interaction probabilities between I plants and J pollinators equal $1/IJ$. The 21 models described above represent all non-redundant combinations of predictor variables based on the species abundance, phenology and morphology data collected.

Network analyses

We summarized network structure using several widely used metrics: connectance, nestedness, network-level specialization index, resource range and paired difference index. Connectance is the proportion of all possible links in the network that are actually observed ($C = I / (A \times P)$) (Yodzis 1980). Nestedness describes the extent to which more specialized species interact with a proper subset of the species generalists interact with, and was calculated using the *NODF* and *WNODF* metrics (Almeida-Neto et al. 2008, Almeida-Neto and Ulrich 2011). Both vary between 0 and 100, with 0 indicating randomly distributed interactions, and 100 indicating perfect nestedness, but *WNODF* uses quantitative rather than qualitative data (interaction frequency, rather than presence/absence), weighting the nestedness contribution of different observations according to their frequencies. Network-level specialization index (H_2) describes the extent to which species observed interaction frequencies deviate from those expected given the distribution of total observation frequencies, and ranges between 0 and 1, where higher values indicate greater reciprocal specialization (Blüthgen et al. 2006). Resource range (RR) and paired difference index (PDI) are measures of species' resource use specificity utilizing qualitative and quantitative interaction data respectively. RR estimates resource use specificity for each species as the fraction of resources used. PDI calculates average difference between a species' highest interaction strength with those of all other available resources (Poisot et al. 2011, 2012). We calculated abundance-weighted means to summarize RR and PDI for the overall network. RR and PDI have been shown to be statistically robust measures of resource use specialization, with higher signal to noise ratio than other common specialization metrics (Poisot et al. 2011, 2012). We performed all calculations in R ver. 2.15.1 using the 'bipartite' (Dormann et al. 2008), 'vegan' (Oksanen et al. 2012) and 'ESM' (Poisot 2011) statistical packages.

We evaluated the ability of each model to predict these aggregate network metrics and pairwise interaction frequencies using Monte Carlo (MC) methods and a likelihood analysis, respectively. Describing counts of observed visits between plants and pollinators as a Poisson process is, in principle, most appropriate for statistical analyses (e.g. we observed counts over a specified area, and marginal sums are not known before the study) (McCullagh and Nelder 1989). However, there were severe problems fitting a Poisson distribution to the data. Consequently, we

modeled species interaction probabilities using the multinomial distribution (after Vázquez et al. 2009b), which better reflected the link between interaction probability and frequency, but represented an ad hoc compromise on the choice of error distribution.

The MC algorithm generated 1000 random quantitative networks in which the same total number of observations present in the observed network were drawn from a multinomial distribution according to the interaction probabilities estimated by each model, with the constraint that marginal sums were non-zero (ensuring consistent network size; R functions used in the MC analysis are available in Supplementary material Appendix 3, and on the Dryad Digital Repository <<http://doi.org/10.5061/dryad.7st32>>). We calculated network metrics for each random network, creating an expected distribution for these metrics against which we compared the observed values for the Mt Murray network. Observed values that fall within the 95% confidence interval for the MC metric distribution provide evidence that the biological mechanisms generating the interaction probabilities for that model generate randomized networks with structural properties similar to the observed network.

Each model's ability to predict pairwise interaction frequencies comprising the observed network can be evaluated by calculating the multinomial log-likelihood of the observed interaction network given the probability matrix defined by each model (after Vázquez et al. 2009b). We calculated Akaike's information criterion (AIC) for each model ($AIC = -2l_i + 2k$, where l_i is the log-likelihood of model i , and k is the number of parameters estimated from the data that were used to calculate pairwise probabilities). For example, model N requires relative abundances calculated from the marginal sums of the interaction network for pollinators (but not for plants), requiring $k = A - 1$ parameters to be estimated from the data. To compare the relative fit of competing models, we calculated ΔAIC values as the difference between the AIC for the best fitting model and each alternative model.

Incomplete sampling

We estimated sampling completeness for both species and interaction in the network using species and interaction accumulation curves, and the Chao2 estimator of asymptotic species richness (Chao et al. 2009). We estimated the accumulation of plant and pollinator species richness as sampling effort was increased using visitation data. To estimate the sampling completeness of interactions in the aggregate network, we estimated the accumulation of unique pairwise interactions, again using visitation data. For plants, we also estimated the accumulation of species richness as sampling effort increased using plant density quadrat data. We calculated the Chao2 estimator of asymptotic species and interaction richness for each of the above analyses.

To evaluate the effect of incomplete sampling of species and interactions on the likelihood analysis, we examined the performance of the interaction probability models on rarefied subsets of the original data set. We rarefied data by randomly removing observed visits from the original visitation dataset. We then constructed interaction network resulting

from the rarefied data, and re-ran the likelihood analysis for this rarefied network. We repeated this procedure 30 times at five levels of observation removal (10, 20, 30, 40 and 50% of observations removed). To help visualize changes in relative and absolute model fit, and to avoid complications from changes in the identity of the best fitting model to different data subsets, ΔAIC s were calculated as the difference in AIC between each model, and the interaction network fitted to itself. Overall model performance is summarized as the mean of the 30 ΔAIC values calculated at each level of observation removal. Model comparisons using AIC are only valid between models fit to the same data. Therefore, ΔAIC values were always calculated using only the AIC values for models fit to the same rarefied subset of the full dataset.

Results

Network description

We documented $n = 900$ visits distributed among $I = 323$ interactions between $P = 41$ plant species and $A = 125$ pollinator morphospecies, resulting in a total of $S = 166$ species, and a network size of $A \times P = 5125$ possible interactions. The 41 plant species observed through interaction sampling belonged to 37 genera from 18 families. *Valeriana sitchensis* had the greatest floral density and was also the most frequently visited plant species at the site (52.4 flowers m^{-2} , 230 visits). Diptera dominated the pollinator community both numerically and in terms of species richness, accounting for 722 observed visits (80%), 245 observed interactions (76%), and 94 observed morphospecies (75%). Of the 94 dipteran morphospecies, 85 (90%) belonged to three families: Anthomyiidae (36), Syrphidae (27), and Muscidae (22). Hymenoptera were the next most common pollinator order, with 171 visits, 61 links, and 12 morphospecies, of which bumble bees were the most common (109 visits, 37 links, 5 species), followed by the sawfly genus *Macrophya* (45 visits, 14 links, 4 morphospecies).

The interaction network was sparse ($C = 0.06$), with a weakly nested topology that was significantly less nested than expected when compared to a null model holding marginal totals the same ($NODF = 19.26$, $Z = -8.2$, $P = 0.01$; $WNODF = 9.59$, $Z = -8.3$, $P = 0.01$; null networks generated using 'r2dtable' method in the 'Vegan' R package). Species interactions were not strongly specialized, but were significantly more specialized than expected given the distribution of their observation frequencies ($H_2 = 0.34$; mean \pm SD of null model: 0.11 ± 0.01). In contrast to H_2 , species resource use was quite specific across the whole interaction network when calculated using either RR or PDI (RR = 0.96; PDI = 0.97). However, these high resource use specificity values are similar to values calculated for other published networks (Poisot et al. 2011). The observed values for all aggregate metrics generally fell near the means of the distributions of values calculated from previously published plant-pollinator interaction networks (Olito unpubl., Poisot et al. 2011).

Network analyses

No interaction probability model generated distributions of all five metrics for which the 95% confidence intervals

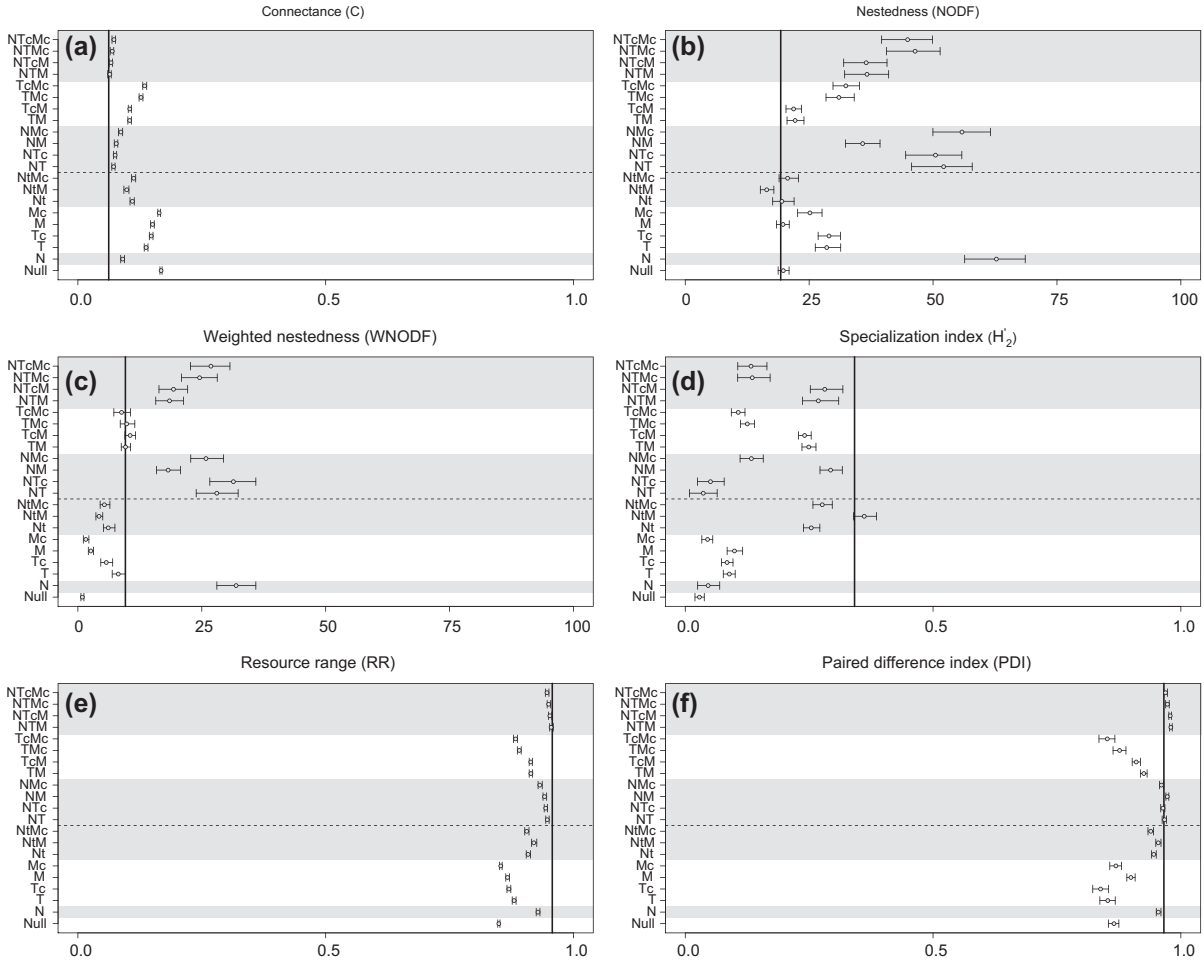


Figure 1. Aggregate network statistics of randomized interaction matrices generated by each of the interaction probability models (estimate \pm monte carlo 95% CI) compared to the estimates for the observed network (vertical black lines). For ease of comparison, models incorporating abundance data appear in grey boxes, with those incorporating **Nt** presented below the horizontal dotted line in the middle box.

included the values for the observed network, but all network metric values for the observed network were accurately predicted by at least one model (Fig. 1a–f). Model **NTM** correctly predicted two out of six metrics (*C* and *RR*), while all other models correctly predicted only one. Thus, different properties of network structure were effectively reproduced by the MC analysis using interaction probabilities derived from all three sources of data, but no single combination of species abundance and biological constraint data appears to reproduce overall network topologies effectively that closely resemble the observed network.

In the likelihood analysis, the phenology model **T** provided the best fit to the observed species pairwise interaction frequencies. Model **Tc** was the next best fitting model, but had an extremely large Δ AIC of 1200.9 (see Table 2 for full likelihood results), indicating that model **T** provided the best fit to the data by a considerable margin. However, model **T** was still a poor predictor of species pairwise interaction frequencies, often assigning high probabilities to interactions that were never observed (Fig. 2a), while many interactions with intermediate to large probabilities had larger observed interaction frequencies than expected given the total number of observations *n* (Fig. 2b).

Incomplete sampling

Interactions were under-sampled in the network (Supplementary material Appendix 4 Fig. A1a). The 319 unique interactions observed represented only 37% of the estimated interaction richness ($S_e = 888$) based on the Chao2 estimator of species richness (Chao et al. 2009), calculated for interactions. This is primarily a consequence of the fact that pollinator species were strongly under-sampled. The 125 pollinator species sampled represent approximately 53.5% of the Chao2 estimate of asymptotic species richness ($S_e = 237.3$), indicating that the pollinator community was severely under-sampled (Supplementary material Appendix 4 Fig. A1b). Quadrat sampling resulted in more complete sampling of the plant community than visitation sampling. Plant species richness was $S_o = 43$ using visitation sampling, representing 80.9% of the estimated $S_e = 53.1$ species (Supplementary material Appendix 4 Fig. A1c), while plant species richness was $S_o = 50$ using quadrat data, which represented 99.8% of the estimated species richness $S_e = 50.1$ species (Supplementary material Appendix 4 Fig. A1d).

The relative ability of the different models to correctly predict species interaction frequencies was generally robust

Table 2. Likelihood analysis results.

Model	k	ΔAIC
T	31	0
Tc	250	1200.91
N	124	1468.94
Nt	31	1497.73
TMc	37	2312.81
NT	155	2590.36
NTc	374	2648.20
NULL	1	3071.29
TcMc	256	3524.46
TM	37	3571.07
NMc	130	3634.77
NtMc	37	3654.79
NTMc	161	4579.95
TcM	256	4684.11
NTcMc	380	4743.43
NtM	37	5276.58
NM	130	5331.30
Mc	6	5448.71
NTM	161	6364.78
NTcM	380	6460.18
M	6	6559.07

to incomplete sampling. Here we report only the results for the four best fitting models (**T**, **Tc**, **N**, **Nt**) and the **NULL** model (Fig. 3). The phenology model **T** always fit the data best as more observations were excluded from the analysis, and model **Tc** was always the second-best fitting model. As more observations were dropped from the analysis, the seasonal relative abundance model **N** gave increasingly poorer fit relative to the other models, and the rank order of model fit between **N** and the daily relative abundance model **Nt** were reversed once 10% or more of the observations were excluded.

Discussion

Predicting network metrics

Metrics of network structure are insensitive to changes in species interactions. There are many possible configurations of species interactions that would, for example, give the same

value of connectance, nestedness, and other metrics. Due to this insensitivity, these metrics are relatively easy to predict, even by models that fail to capture the true underlying determinants of network structure (Fox 2006). Every aggregate network metric we tested was correctly predicted by at least one of the interaction probability models in the MC analysis (Fig. 1). Furthermore, the models that best predicted network metrics often predicted pairwise interaction frequencies especially poorly (Table 2), suggesting that they predict network structural metrics at the cost of actually misdescribing the underlying determinants of network structure. Correctly predicting species interaction frequencies is a non-trivial goal for statistical models of network structure. However, models that can correctly predict metrics while incorrectly predicting interactions provide little information about the biological mechanisms shaping network structure.

Even network metrics based on quantitative data, such as *WNODF*, H_2 and PDI set the predictive bar too low for network models. PDI has been shown to be a robust measure of species resource use specificity, with a high signal to noise ratio (Poisot et al. 2012). Its predictability by several models that performed poorly in the likelihood analysis demonstrates that a variety of mechanisms that correspond poorly to species' actual resource use can still predict statistically indistinguishable levels of specificity. Metrics of species resource use specificity may provide evidence of adaptive specialization (Poisot et al. 2011), but our results suggest that this interpretation is not always correct. Similarly, H_2 is purportedly more robust to variable interaction frequencies and sampling biases than other metrics of species specialization, because it accounts for the distribution of total species observation frequencies (Blüthgen et al. 2006, 2008). However, model **NtM** accurately reproduced the H_2 of the observed network in the MC analysis, despite predicting pairwise interaction frequencies worse than the **NULL** model (Fig. 1e, Table 2). This outcome is a consequence of how H_2 is calculated, and highlights potential difficulties interpreting H_2 in the context of biological constraints. H_2 quantifies the deviation of observed interaction frequencies from those expected given the distribution of interaction frequencies and neutral interactions. Therefore, if species interactions are influenced by traits that do not co-vary strongly with interaction frequency, any resulting networks will

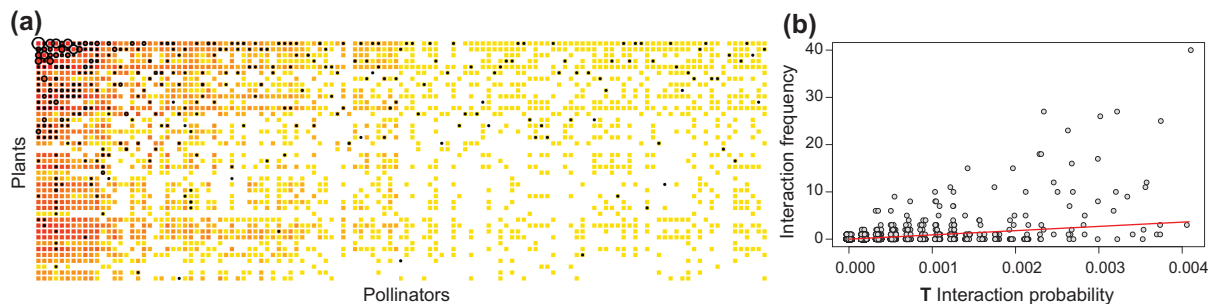


Figure 2. Fit of model **T** to the observed interaction network. (a) The observed interaction network (black circles, sizes scaled to interaction frequency) is overlain on a color visualization of the corresponding interaction probabilities predicted by the model. The color spectrum represents a color axis of the log transformed species pairwise interaction probabilities predicted by the model. Circles overlaid on red-orange grid cells indicate better model fit, while empty red-orange cells or circles on white-yellow cells indicate poor fit. (b) The relation between observed species pairwise interaction frequencies (grey points) and species pairwise interaction probabilities predicted by model **T**. The expected interaction frequencies (red line) were calculated as the product of the predicted interaction probabilities and the total number of observations n .

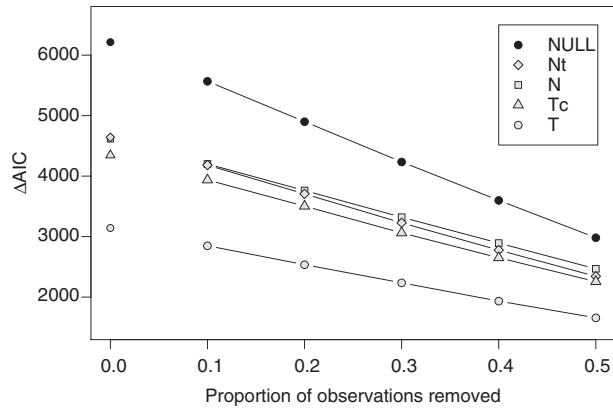


Figure 3. Effects of incomplete sampling on the performance of the three best fitting models compared to the null model (solid black line). Leftmost points indicate the ΔAIC values for the four models when calculated for the full dataset, and represent single values. Points and lines indicate mean ΔAIC values for each model calculated from 30 replicate rarefied subsets of the original data set. Note that log-likelihoods necessarily decrease as more observations are removed from the analysis, and the ΔAIC values reflect this.

by definition be more specialized than expected given the distribution of interaction frequencies. Inferring tighter co-evolutionary history between mutualists from H_2 (Blüthgen et al. 2008) or related metrics (e.g. d') should be done cautiously, as this is just one possible explanation for weak or non-existent relations between interaction frequency and traits influencing biological constraints.

Mechanisms for network structure

All of the models tested left a large amount of variation in pairwise interaction frequencies unexplained in the likelihood analysis (Table 2). For example, the best fitting model (T) generally did a better job of predicting rare interactions than common ones (Fig. 2a–b). Model T correctly assigned low probabilities to many unobserved or rare interactions (Fig. 2a, white/yellow cells), but also assigned high probabilities to many unobserved interactions (Fig. 2a, orange/red cells). At the same time, many interactions with intermediate to large probabilities had larger observed interaction frequencies than expected given the total number of observations n (Fig. 2b). To some extent, the inability of the models to correctly predict interaction frequencies is not surprising. Recent work suggests that single traits can sometimes predict a large amount of network structure, but more traits are often needed to fully describe all of the interactions in a network (< 10 dimensions; Eklöf et al. 2013). However, in contrast to previous studies (Stang et al. 2007, Vázquez et al. 2009b, Ibanez 2012, Eklöf et al. 2013, Canard et al. 2014, Vizentin-Bugoni et al. 2014), phenology alone was the best predictor of observed species pairwise interaction frequencies, and several single-term models outperformed more complicated (more traits) ones. Many alpine plant and pollinator species are sensitive to snowmelt, temperature, and photoperiod cues to initiate development, reproduction, and activity (Kudo 2006, Forrest and Thomson 2011). Consequently, there is broad scope for temporal variation in abiotic conditions to influence species interaction frequencies

at multiple time scales. For example, several common early-season plant species at the Mt Murray field site (e.g. *Erythronium grandiflorum* and *Claytonia lanceolata*) begin flowering shortly after snowmelt (Thomson et al. 1994, Moss 2008), and are therefore unlikely to interact with pollinator species that undergo a period of larval feeding to offset the energetic costs of metamorphosis (e.g. many Anthomyiidae and Muscidae, Hockett and Vockeroth 1987). At shorter timescales, the dynamic abiotic conditions typical of alpine environments may disrupt otherwise favorable interactions and strongly influence who interacts with whom. At Mt Murray, pollinators experienced only two periods of three or more consecutive days in which the weather was favourable for flight, and were often not observed immediately after a storm, leaving gaps in their phenologies lasting longer than the poor weather (Olito unpubl.). Under these conditions, the identity of pollinators that happen to be active, not only during the same period of the season, but on specific favourable days, may have a strong influence on species interaction frequencies.

Possibly, models incorporating more detailed predictor variables could better predict species interaction frequencies. The low-resolution size classes we used most likely do not adequately capture morphological constraints on who can pollinate whom (Stang et al. 2007). However, if morphological trait matching was an important biological constraint in this system, even low-resolution morphology data should have at least marginally improved model fit, but did not (Table 2). It seems more likely that the models' failures were a result of alternative mechanisms playing an important role in determining species interaction frequencies.

Aspects of pollinator foraging biology not included in this study may be important determinants of network structure. For example, anthophilous insects make foraging decisions based on a variety of floral attractive cues not included in this study, including color, and floral and nectar chemistry (Gegear et al. 2007, Irwin et al. 2010, Junker et al. 2010, 2013). The spatial scale of network sampling will also determine the identity and relative importance of underlying mechanisms. Pollinators make foraging decisions based on resource availability at multiple spatial scales (Thomson 1981, Real 1981, Harder and Real 1987), which can influence both local relative abundances, and observed interaction frequencies.

Other aspects of pollinator biology, unrelated to foraging and not previously examined in network models, may also play an important role in determining species interaction frequencies. Anthomyiid and muscid flies were the most diverse and numerous pollinators at the Mt Murray field site, but their high interaction frequencies were largely attributable to their reproductive, rather than foraging, biology. Many species in both families were observed to contact plant reproductive structures while participating in mating swarms on target plant species (Olito unpubl.; also see Hockett and Vockeroth 1987), resulting in high interaction frequencies that were not determined by morphological or energetic considerations relevant to foraging. Females of some of these species also visit flowers to oviposit, a behavior that can facilitate pollination, but also subsequent seed predation by developing larvae. Such mutualistic-antagonistic interactions can have non-trivial effects on plant and

pollinator reproduction, leading to ecological and evolutionary dynamics that are still poorly understood (Pellmyr and Huth 1994, Bronstein 2001). Predicting interactions for many dipterans, which dominate alpine and high latitude pollinator faunas (Kevan 1972, Kearns 1992) will require a more complete understanding of both their foraging and reproductive biology.

Incomplete sampling

Pollinator species were considerably under-sampled for this study, and so rare interactions were under-sampled as well (Supplementary Material Appendix 4 Fig. A1a–b). Under-sampling both species and interactions not only results in underestimates of network size, it can also bias the estimation of other network structural metrics (Blüthgen et al. 2008, Almeida-Neto and Ulrich 2011) and the likelihood analysis. However, the relative performance of the different models at predicting the observed interaction network were robust to incomplete sampling (Fig. 3), and highlight the importance of phenology in determining species interactions in this network. The phenology model **T** was always the best predictor of species interaction frequencies, even as a greater proportion of observations were randomly excluded from the analysis (Fig. 3). Interestingly, seasonal relative abundances (**N**) became a poorer predictor of species interaction frequencies than daily relative abundances (**N_t**) once 10% or more of the observations were excluded. The ability of neutrality models to predict network structure should be interpreted cautiously, particularly when species abundances are estimated from interaction data for under-sampled species (Canard et al. 2014, Vizentin-Bugoni et al. 2014). Excluding interactions also decreases variation in species abundances, and thus the likelihood of detecting an effect of abundance on interaction frequency (although this has a similar effect on species phenologies). Because rare species are expected to have fewer interactions under the neutrality hypothesis it is impossible to definitively tease apart sampling biases from neutrality without demonstrating that the probability of detecting interactions for rare species is lower than for common ones (Dorado et al. 2011). Given the wide acceptance of neutral models to explain network structure, future studies should carefully examine this assumption.

Conclusions

The relative importance of different mechanisms contributing to mutualistic network structure depends on what structural aspects are being predicted. Seemingly plausible models of network structure that are in fact poor predictors of species interactions can spuriously predict commonly used metrics, including those based on quantitative data. However, it is species interactions that determine reproductive success (especially for plants), and therefore the ecological and evolutionary dynamics of plant-pollinator mutualistic communities. Future progress in understanding the structure and dynamics of mutualistic networks will require new approaches and metrics that are more sensitive to small changes in network structure, emphasize accurate prediction of interactions, and better reflect the biology underlying species interactions. Ideally, these approaches

should also account for the effects of under-sampling species and interactions, particularly studies evaluating abundance-based models of network structure. The broad scope for phenology to influence network structure in both alpine and desert (Vázquez et al. 2009b) habitats, and the ease with which it can be incorporated into network sampling designs makes documentation and analysis of temporally explicit networks a natural first step for future studies.

Acknowledgements – Author contributions: CO designed the study with advice from JWF. CO collected the data and performed the analyses. CO wrote the manuscript with assistance from JWF, L. D. Harder, S. Hausch, B. Kopach, G. Legault, J. Mee, C. A. Olito and C. Venables provided helpful comments and discussions. J. Acorn, G. Ball, G. Anweiler, L. D. Harder, R. Lem, A. Pierce, G. Pohl, J. Savage and D. Shpeley, assisted with species identifications. J. Swann and the Univ. of Calgary Invertebrate Museum provided materials, patience and expert advice regarding insect identifications. Comments from T. Poisot greatly improved the study and manuscript. This research was funded by an NSERC of Canada Discovery Grant to JWF, as well as Graeme Bell and Norma Kay Sullivan-Bell and Queen Elizabeth II Graduate Scholarships awarded to CO.

References

- Almeida-Neto, M. and Ulrich, W. 2011. A straightforward computational approach for measuring nestedness using quantitative matrices. – *Environ. Model. Software* 26: 173–178.
- Almeida-Neto, M. et al. 2008. A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. – *Oikos* 117: 1227–1239.
- Blüthgen, N. et al. 2006. Measuring specialization in species interaction networks. – *BMC Ecol.* 6: 9.
- Blüthgen, N. et al. 2008. What do interaction network metrics tell us about specialization and biological traits? – *Ecology* 89: 3387–3399.
- Bronstein, J. L. 2001. The exploitation of mutualisms. – *Ecol. Lett.* 4: 277–287.
- Canard, E. F. et al. 2014. Empirical evaluation of neutral interactions in host–parasite networks. – *Am. Nat.* 183: 468–479.
- Chao, A. et al. 2009. Sufficient sampling for asymptotic minimum species richness estimators. – *Ecology* 90: 1125–1133.
- Dorado, J. et al. 2011. Rareness and specialization in plant–pollinator networks. – *Ecology* 92: 19–25.
- Dormann, C. F. et al. 2008. The bipartite package, ver. 0.5. R Project for Statistical Computing, Vienna, Austria. <www.R-project.org>.
- Dupont, Y. L. et al. 2009. Spatio-temporal variation in the structure of pollination networks. – *Oikos* 118: 1261–1269.
- Eklöf, A. et al. 2013. The dimensionality of ecological networks. – *Ecol. Lett.* 16: 577–583.
- Forrest, J. R. K. and Thomson, J. D. 2011. An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. – *Ecol. Monogr.* 81: 469–491.
- Fox, J. W. 2006. Current food web models cannot explain the overall topological structure of observed food webs. – *Oikos* 115: 97–109.
- Gegear, R. J. et al. 2007. Ecological context influences pollinator deterrence by alkaloids in floral nectar. – *Ecol. Lett.* 10: 375–382.
- Harder, L. D. and Real, L. 1987. Why are bumble bees risk averse? – *Ecology* 68: 1104–1108.
- Hegland, S. J. et al. 2010. How to monitor ecological communities cost-efficiently: the example of plant–pollinator networks. – *Biol. Conserv.* 143: 2092–2101.

- Huckett, H. C. and Vockeroth, J. R. 1987. Muscidae. – In: McAlpine et al. (eds), Manual of Nearctic Diptera vol. 1115–1131. Res. Branch Agric. Canada Monogr. No. 28. Biosystematics Research Centre, Ottawa, ON, Canada.
- Ibanez, S. 2012. Optimizing size thresholds in a plant–pollinator interaction web: towards a mechanistic understanding of ecological networks. – *Oecologia* 170: 223–242.
- Irwin, R. E. et al. 2010. Nectar robbing: ecological and evolutionary perspectives. – *Annu. Rev. Ecol. Evol. Syst.* 41: 271–292.
- James, A. et al. 2012. Disentangling nestedness from models of ecological complexity. – *Nature* 487: 227–230.
- Jordano, P. 1987. Patterns of mutualistic interactions in pollination and seed dispersal: connectance, dependence asymmetries, and coevolution. – *Am. Nat.* 129: 657–677.
- Jordano, P. et al. 2003. Invariant properties in coevolutionary networks of plant–animal interactions. – *Ecol. Lett.* 6: 69–81.
- Jordano, P. et al. 2006. The ecological consequences of complex topology and nested structure in pollination webs. – In: Waser, N. M. and Ollerton, J. (eds), Plant–pollinator interactions: from specialization to generalization. Univ. of Chicago Press.
- Junker, R. R. et al. 2010. Responses to olfactory signals reflect network structure of flower–visitor interactions. – *J. Anim. Ecol.* 79: 818–823.
- Junker, R. R. et al. 2013. Specialization on traits as basis for the niche-breadth of flower visitors and as structuring mechanism of ecological networks. – *Funct. Ecol.* 27: 329–341.
- Kallimanis, A. S. et al. 2009. Do plant–pollinator interaction networks result from stochastic processes? – *Ecol. Modell.* 220: 684–693.
- Kay, K. and Schemske, D. 2004. Geographic patterns in plant–pollinator mutualistic networks: comment. – *Ecology* 85: 875–878.
- Kearns, C. 1992. Anthophilous fly distribution across an elevation gradient. – *Am. Midl. Nat.* 127: 172–182.
- Kevan, P. G. 1972. Insect pollination of high arctic flowers. – *J. Ecol.* 60: 831–847.
- Kudo, G. 2006. Flowering phenologies of animal-pollinated plants: reproductive strategies and agents of selection. – In: Harder, L. D. and Barrett, S. (eds), Ecology and evolution of flowers. Oxford Univ. Press, pp. 102–119.
- McCullagh, P. and Nelder, J. A. 1989. Generalized linear models, 2nd edn. – Chapman and Hall/CRC.
- Moss, E. H. 2008. Flora of Alberta, 2nd edn. Revised by J.G. Packer. – Univ. of Toronto Press.
- Oksanen, J. et al. 2012. Vegan: community ecology package. – R package ver. 2.0-4. <<http://CRAN.R-project.org/package=vegan>>.
- Pellmyr, O. and Huth, C. J. 1994. Evolutionary stability of mutualism between yuccas and yucca moths. – *Nature* 372: 257–260.
- Poisot, T. 2011. ESM: ecological specificity measures. R package ver. 2.0.3-02/r47. <<http://R-Forge.R-project.org/projects/esm/>>.
- Poisot, T. et al. 2011. A conceptual framework for the evolution of ecological specialization. – *Ecol. Lett.* 14: 841–851.
- Poisot, T. et al. 2012. A comparative study of ecological specialization estimators. – *Meth. Ecol. Evol.* 3: 537–544.
- Real, L. A. 1981. Uncertainty and pollinator–plant interactions: the foraging behavior of bees and wasps on artificial flowers. – *Ecology* 62: 20–26.
- Rivera-Hutinel, A. et al. 2012. Effects of sampling completeness on the structure of plant–pollinator networks. – *Ecology* 93: 1593–1603.
- Ságyo, R. et al. 2013. Evaluating factors that predict the structure of a commensalistic epiphyte–phorophyte network. – *Proc. R. Soc. B.* 280: 20122821.
- Santamaria, L. and Rodriguez-Girones M. A. 2007. Linkage rules for plant–pollinator networks: trait complementarity or exploitation barriers? – *PLoS Biol.* 5: 354–362.
- Stang, M. et al. 2007. Asymmetric specialization and extinction risk in plant–flower visitor webs: a matter of morphology or abundance? – *Oecologia* 151: 442–453.
- Stang, M. et al. 2009. Size-specific interaction patterns and size matching in a plant–pollinator interaction web. – *Ann. Bot.* 103: 1459–1469.
- Strauss, S. and Irwin, R. 2004. Ecological and evolutionary consequences of multispecies plant–animal interactions. – *Annu. Rev. Ecol. Evol. Syst.* 35: 435–466.
- Thomson, J. 1981. Spatial and temporal components of resource assessment by flower-feeding insects. – *J. Anim. Ecol.* 50: 49–59.
- Thomson, J. D. et al. 1994. Pollen viability, vigor, and competitive ability in *Erythronium grandiflorum* (Liliaceae). – *Am. J. Bot.* 81: 1257–1266.
- Vázquez, D. P. and Aizen, M. 2004. Asymmetric specialization: a pervasive feature of plant–pollinator interactions. – *Ecology* 85: 1251–1257.
- Vázquez, D. P. et al. 2005. Interaction frequency as a surrogate for the total effect of animal mutualists on plants. – *Ecol. Lett.* 8: 1088–1094.
- Vázquez, D. P. et al. 2007. Species abundance and asymmetric interaction strength in ecological networks. – *Oikos* 116: 112–127.
- Vázquez, D. P. et al. 2009a. Uniting pattern and process in plant–animal mutualistic networks: a review. – *Ann. Bot.* 103: 1445–1457.
- Vázquez, D. P. et al. 2009b. Evaluating multiple determinants of the structure of plant–animal mutualistic networks. – *Ecology* 90: 2039–2046.
- Verdú, M. and Valiente-Banuet, A. 2011. The relative contribution of abundance and phylogeny to the structure of plant facilitation networks. – *Oikos* 120: 1351–1356.
- Vermaat, J. E. et al. 2009. Major dimensions in food-web structure properties. – *Ecology* 90: 278–282.
- Vizentin-Bugoni, J. et al. 2014. Processes entangling interactions in communities: forbidden links are more important than abundance in a hummingbird–plant network. – *Proc. R. Soc. B* 281: 20132397.
- Waser, N. et al. 1996. Generalization in pollination systems, and why it matters. – *Ecology* 77: 1043–1060.
- Yodzis, P. 1980. The connectance of real ecosystems. – *Nature* 284: 544–545.

Supplementary material (available online as Appendix oik.01439 at <www.oikosoffice.lu.se/readers/appendix>). Appendix 1–4.